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(54) Title: ANTIVIRAL THERAPY USE OF P-GLYCOPROTEIN MODULATORS

(57) Abstract: The present invention relates to a pharmaceutical composition comprising a 10, 11 methanodibenzosuberane and use thereof for the treatment of HIV infection. Co-administration of the 10, 11 methanodibenzosuberane with an HIV protease inhibitor increases the concentration of the protease inhibitor in certain tissues, including the brain and testes, without substantially increasing plasma levels of the protease inhibitor. Accordingly, additional antiviral therapy can be achieved without use of increased drug dosages, thereby reducing the potential for occurrence of undesirable side effects deriving from drug toxicity.

## ANTIVIRAL THERAPY USE OF P-GLYCOPROTEIN MODULATORS

### Field of the Invention

The present invention relates to treatment of viral infections. More particularly the present invention is directed to the use of certain P-glycoprotein modulators to increase the concentration of HIV-protease inhibitors in certain tissues.

### Background and Summary of the Invention

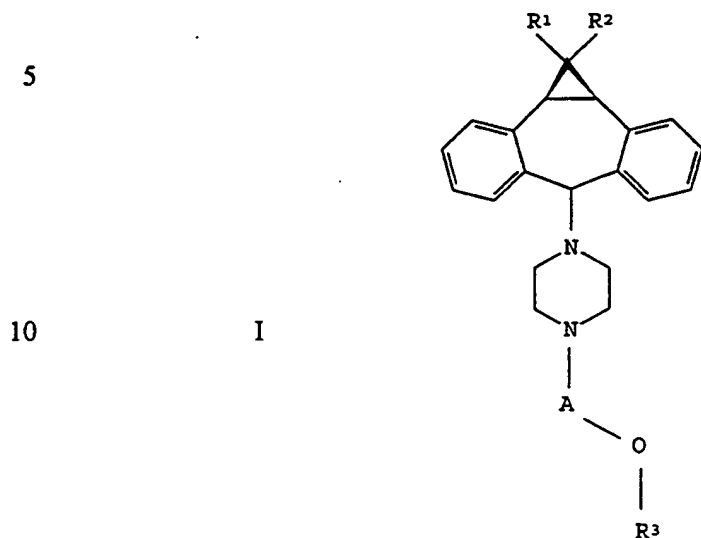
HIV protease inhibitors have proven to be effective in the treatment of HIV-1 infection. However, the utility of such drugs can be limited due to poor transport across certain biological membranes. Oral absorption of protease inhibitors is often low and variable, and penetration into certain tissues, including the brain and testes, is often poor. The resultant non-uniform distribution of the antiviral drug in the body leaves certain tissues as sanctuaries for viral proliferation.

P-glycoprotein is an ATP dependent efflux membrane transporter with broad substrate specificity for a variety of structurally diverse drugs. P-glycoprotein is distributed in various normal tissues, including, of particular importance in drug disposition, epithelial cells in the gastrointestinal tract, the liver, and the kidney. Apical expression of P-glycoprotein in such tissues results in reduced absorption (gastrointestinal tract), and enhanced elimination into the bile (liver) and urine (kidney) for drugs functioning as P-glycoprotein substrates. In addition, expression of P-glycoprotein at the level of the blood-brain barrier has been shown to be a critical factor in preventing the entry of some drugs into the central nervous system. Previous work has shown that various HIV-1 protease inhibitors are substrates of P-glycoprotein, explaining some of the limits on membrane permeability of these drugs. See, for example, Kim, R.B., et al., The Drug Transporter P-Glycoprotein Limits Oral Absorption and Brain Entry of HIV-1 Protease Inhibitors, J. Clin. Invest., 101:289-294, 1998.

Certain 10,11-methanodibenzosuberane derivatives have been shown to be pharmaceutically active agents in the treatment of multidrug resistance in cancer therapy. See, for example, U.S. Patents Nos. 5,654,304 and 5,874,434. Such compounds are known to interact with P-glycoprotein.

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The present invention relates to a use of a 10,11-methanodibenzosuberanes of formula (I):



15 wherein: A is  $-\text{CH}_2\text{CH}_2-$ ;  $-\text{CH}_2\text{CHR}^a\text{CH}_2-$  where  $\text{R}^a$  is H, OH or lower acyloxy; or  $-\text{CH}_2\text{CHR}^b\text{CHR}^c\text{CH}_2-$  where one of  $\text{R}^b$  or  $\text{R}^c$  is H, OH, or lower acyloxy, and the other is H;

$\text{R}^1$  is H, F, Cl, or Br;

$\text{R}^2$  is H, F, Cl, or Br; and

20  $\text{R}^3$  is heteroaryl or phenyl optionally substituted with F, Cl, Br,  $\text{CF}_3$ , CN,  $\text{NO}_2$ , or  $\text{OCHF}_2$ ; or a pharmaceutically acceptable salt thereof; for the manufacture of a medicament for the treatment of HIV in a patient undergoing treatment with an HIV protease inhibitor. The use increases the concentration of the HIV inhibitor in the brain and/or testes of the patient without significantly increasing plasma levels of the protease inhibitor. Accordingly, more effective antiviral therapy

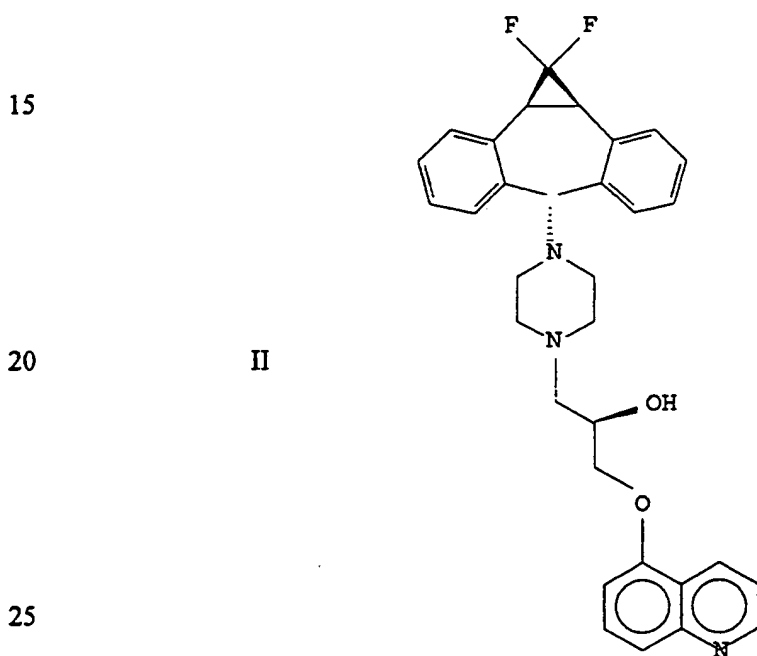
25 can be achieved without use of increased drug dosages, thereby reducing the potential for occurrence of undesirable side effects deriving from drug toxicity. Thus, one aspect of this invention relates to a method for increasing the concentration of an HIV protease inhibitor in the brain of a patient, the method comprising administering to an

30 HIV infected patient an amount of a 10,11-methanodibenzosuberane of formula (I), or a pharmaceutically acceptable salt thereof, and co-administering to the patient a therapeutically effective amount of the protease inhibitor.

Another related aspect of this invention is a method of treatment of an HIV infected patient. The method comprises administering a compound comprising a 10,11-methanodibenzosuberane of formula (I) in an amount effective to increase the concentration of a co-administered protease inhibitor in the brain and testes of the patient.

In another embodiment, the 10,11-methanodibenzosuberane of formula (I) is administered in combination with a protease inhibitor to increase concentrations of the protease inhibitor in the brain.

Still another aspect of this invention is a pharmaceutical composition comprising a protease inhibitor, most preferably nelfinavir, and a 10,11-methanodibenzosuberane of formula (I), with a pharmaceutical carrier. In a preferred embodiment, the 10,11-methanodibenzosuberane is a compound of formula (II):



Still another aspect of this invention is the use of an HIV protease inhibitor for the manufacture of a medicament for the treatment of HIV wherein the concentration of the protease inhibitor in the brain is increased by co-administration with a 10, 11-methanodibenzosuberane of formula (I), or a pharmaceutically acceptable salt thereof.

Additional features of the present invention will become apparent to those skilled in the art upon consideration of the following detailed description of preferred embodiments exemplifying the best mode of carrying out the invention.

## 5 Brief Description of the Drawings

Fig. 1 is a plot of percent inhibition of P-glycoprotein mediated [<sup>3</sup>H]-digoxin transport across a Caco-2 cell culture monolayer verses concentration of putative inhibitor at varying concentrations of formula (II) (◇), nelfinavir (●), ritonavir (○), saquinavir (■), and indinavir (Δ).

10 Fig. 2 shows tissue levels of [<sup>14</sup>C]-nelfinavir in *mdrla* (+/+) mice given 50 mg/kg of formula (II) (plasma - open symbols, brain - closed symbols) in divided doses 30 min prior to and simultaneously with (5 mg/kg) [<sup>14</sup>C]-nelfinavir (triangles) or vehicle (circles).

Fig. 3 shows the effect of P-glycoprotein inhibitors on tissue:plasma  
15 concentration ratios of [<sup>14</sup>C]-nelfinavir in *mdrla* (+/+) and *mdrla* (-/-) mice.

## Detailed Description of the Invention

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

20 Additional details on the preparation of such compounds, and the meaning and scope of the terminology and definitions thereof, are detailed in U.S. Patent No. 5,654,304.

The term "lower acyloxy" refers to the group —O—C(O)—R' where R' is lower alkyl.

The term "heteroaryl" refers to a monovalent unsaturated aromatic  
25 carbocyclic radical having at least one hetero atom, such as N, O or S, within the ring, such as quinolyl, benzofuranyl and pyridyl.

A "pharmaceutically acceptable salt" may be any salt derived from an inorganic or organic acid. The term "pharmaceutically acceptable anion" refers to the anion of such acid addition salts. The salt and/or the anion are chosen not to be  
30 biologically or otherwise undesirable.

The term "treatment" or "treating" means any treatment of a disease in a mammal, including:

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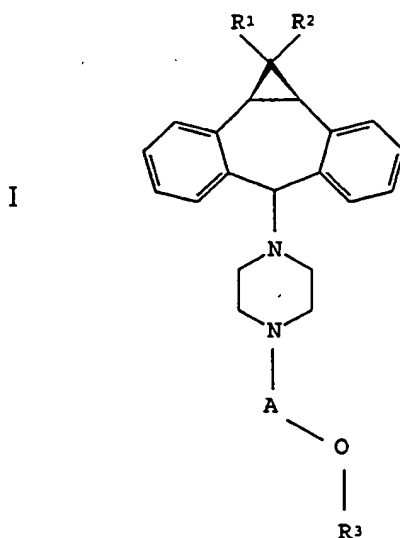
- (i) preventing the disease, that is, causing the clinical symptoms of the disease not to develop;
- (ii) inhibiting the disease, that is, arresting the development of clinical symptoms; and/or
- 5 (iii) relieving the disease, that is, causing the regression of clinical symptoms.

The term "effective amount" means a dosage sufficient to provide treatment for the disease state being treated. This will vary depending on the patient, the disease and the treatment being effected.

10 The term "co-administer" means the administration of more than one active agent as part of the same treatment regimen, whether they are administered simultaneously or at different times.

"Structure of formula (I)" refers to the generic structure of the compounds of the invention.

15 The present invention is a method for increasing the concentration of an HIV protease inhibitor in the brain and testes of a patient, said method comprising administering to an HIV-infected patient an amount of a 10,11-methanodibenzosuberanes of the formula (I):



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wherein: A is  $-\text{CH}_2\text{CH}_2-$ ;  $-\text{CH}_2\text{CHR}^a\text{CH}_2-$  where  $\text{R}^a$  is H, OH or lower acyloxy; or  $-\text{CH}_2\text{CHR}^b\text{CHR}^c\text{CH}_2-$  where one of  $\text{R}^b$  or  $\text{R}^c$  is H, OH, or lower acyloxy, and the other is H;

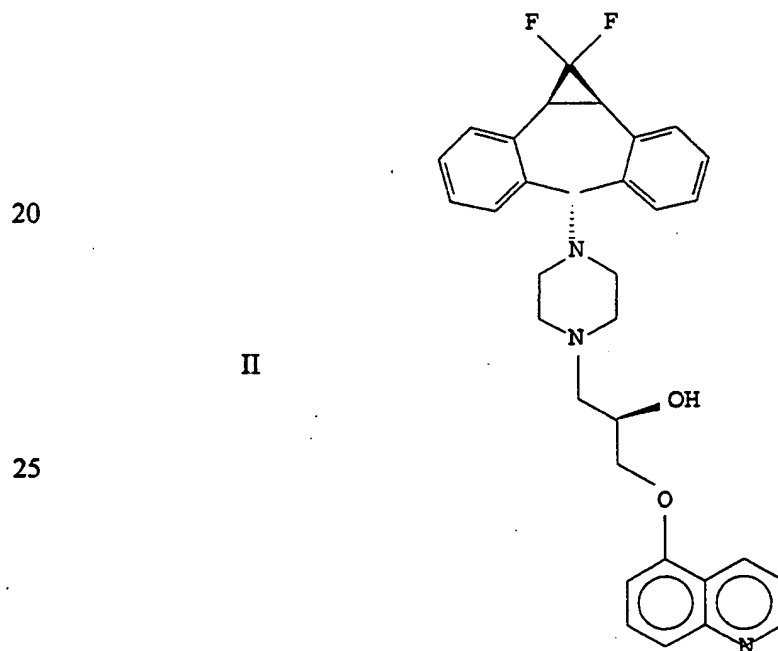
$\text{R}^1$  is H, F, Cl, or Br;

5  $\text{R}^2$  is H, F, Cl, or Br; and

$\text{R}^3$  is heteroaryl or phenyl optionally substituted with F, Cl, Br,  $\text{CF}_3$ , CN,  $\text{NO}_2$ , or  $\text{OCHF}_2$ ; or a pharmaceutically acceptable salts thereof; and co-administering to the patient a therapeutically effective amount to the protease inhibitor.

10 In a preferred embodiment, a compound of formula (I) is used wherein A is  $-\text{CH}_2\text{CHR}^a\text{CH}_2-$ . In another preferred embodiment,  $\text{R}^1$  and  $\text{R}^2$  are F. In still another preferred embodiment,  $\text{R}^3$  is an optionally substituted quinolyl, preferably quinol-5-yl.

In another preferred embodiment of the present invention, a compound  
15 of formula (II):



30 is employed with protease inhibitors in the method of the present invention.

Examples of such protease inhibitors contemplated by the present invention are NELFINAVIR, which is preferably administered as the mesylate salt at



750 mg three times per day (Agouron Pharmaceuticals (La Jolla, CA)) (U.S. Patent No. 5,484,926); RITONAVIR, which is preferably administered at 600 mg twice daily (Roche Ltd. (Lewes, UK) (U.S. Patent No. 5,484,801); SAQUINAVIR, which is preferably administered as the mesylate salt at 1,200 mg three times per day (Roche Discovery (Rahway, NJ)) (U.S. Patent No. 5,196,438); INDINAVIR, which is preferably administered as the sulfate salt at 800 mg three times per day (Merck Research Laboratories) (U.S. Patent No. 5,413,999); and AMPRENAVIR, which is preferably administered at 1,200 mg twice daily (U.S. Patent No. 5,585,397). The skilled artisan would recognize that this list is not exhaustive. Additionally the skilled  
10 artisan would recognize that the protease inhibitor's administration to a patient may vary from the preferred.

The HIV-1 virus enters the brain and other organs such as the testes relatively early after primary infection. Reduction of the viral load in such organs has proven to be difficult, as most of the current HIV antiviral agents do not readily  
15 penetrate into the tissues to provide concentrations effective to prevent viral replication. See Groothuis, D.R., and Levy, R.M., The entry of antiviral drugs into the central nervous system, J. NeuroVirology, 3:387-400, 1997. The low rate of drug transport into these pharmacologic sanctuary sites is the consequence of a functional barrier to drug entry. HIV protease inhibitors have been found to be excellent  
20 substrates for the membrane efflux pump P-glycoprotein, which is localized in the apical domain of capillary endothelial cells of the brain and testis. The P-glycoprotein pump works to limit drug distribution into these tissues. See, for example, Kim, R.B., et al., The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors, J. Clin. Invest., 101:289-294, 1998; Lee, C.G.L., et al.,  
25 HIV-1 protease inhibitors are substrates for the *mdrl* multidrug transporter, Biochemistry, 37:3594-3601, 1998; Kim, A.E., et al., Saquinavir, an HIV protease inhibitor, is transported by P-glycoprotein, J. Pharmacol. Exp. Ther., 286:1439-1445, 1998; Thiebaut, F., et al., Cellular localization of the multidrug resistance gene product P-glycoprotein in normal human tissue, Proc. Natl. Acad. Sci., U.S.A.,  
30 84:7735-7738, 1987; Gordon-Cardo, C., et al., Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites, Proc. Natl. Acad. Sci., U.S.A., 86:695-698, 1989.

The present invention enables pharmacological inhibition of the functional activity of the P-glycoprotein transporter on HIV protease inhibitor substrates through use of a 10,11-methanodibenzosuberane of formula (I) co-administered with an HIV protease inhibitor. Such modulation of P-glycoprotein activity results in significantly enhanced HIV protease inhibitor concentrations in both the brain and testes relative to drug concentration in plasma.

The magnitude of the effect of P-glycoprotein inhibition attainable by administration of the compounds of formula (I) is tissue dependent; for example, the tissue:plasma drug concentration ratio is enhanced in the brain to a greater extent than in the testes. This difference is believed to be related to the level of P-glycoprotein function in the respective tissues. There is about a 30-fold difference in nelfinavir concentration in the brain of *mdrla*(+/+) and *mdrla*(-/-) mice compared to only a 4-fold concentration difference in the testes. P-glycoprotein inhibition using the compounds of formula (I) exhibits similar tissue differences. Notably, however, nelfinavir concentration differences achieved in both organs indicates a 75 to 90% absence of P-glycoprotein function based on comparable data in the *mdrla*(-/-) mice. At the highest doses of the compound of formula (II), the concentrations of nelfinavir in the brain and testes are equal to or higher than the drug concentration in plasma. Co-administration of a 10,11-methanodibenzosuberane of formula (I) with an HIV protease inhibitor in accordance with this invention minimizes P-glycoprotein modulated drug concentration differences between plasma and the brain and testes, thereby reducing or eliminating these tissues as sanctuaries for viral proliferation in patients receiving protease inhibitor therapy.

The present invention provides advantages over use of prior art P-glycoprotein inhibitors such as quinidine, verapamil, valspodar, and cyclosporine A, which are known to interact with drug metabolizing enzymes, in particular, members of the cytochrome P4503A subfamily (CYP3A). Inhibitors of P-glycoprotein are frequently inhibitors of CYP3A and vice-versa. See, for example, Wachter, V.J., et al., Overlapping substrate specificities and tissue distribution of cytochrome P4503A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy, *Mol. Carcinogen*, 13:129-134, 1995; Kim, R.B., et al., Interrelationship between substrates and inhibitors of human CYP3A and P-

glycoprotein, *Pharm. Res.*, 16:408-44, 1999. Accordingly, with drugs such as quinidine, verapamil, valsopodar, and cyclosporine A, a dual interaction occurs whereby reduced P-glycoprotein function is associated with increased plasma levels of the CYP3A substrate.

- 5                   Although many P-glycoprotein inhibitors impair CYP3A-mediated metabolism, this is not an absolute relationship. In fact, the two characteristics appear to be independently determined such that some CYP3A inhibitors do not cause significant impairment of P-glycoprotein function and, more importantly, the reverse situation is possible, i.e., effective transporter inhibition with minimal effect on
- 10 CYP3A. See Wandel, C., et al., P-glycoprotein and cytochrome P4503A inhibition: dissociation of inhibitory potencies, *Cancer Res.*, in press, 1999. The 10,11-methanodibenzosuberanes of formula (I) are representative of such drugs. For example, the affinity of the compound of formula (II) for CYP3A is some 40-fold less than that for P-glycoprotein. Shepard, R.L., et al., Selectivity of the potent P-
- 15 glycoprotein modulator, LY335979, *Proc. Amer. Assoc. Cancer. Res.*, 39:362, 1998; Dantzig, A., *J. Pharmco. Exp. Ther.*, 290:854-862, 1999. This selectivity would account for the relative small formula (II)-induced changes in nelfinavir's plasma level. Thus, the present invention has advantages over prior art P-glycoprotein inhibitors, since systemic toxicity from the antiviral agent would not be expected to
- 20 increase following administration of compounds of formula (I).

- An additional problem associated with prior art use of P-glycoprotein modulators has been their limited potency. Because of this limited potency, effective levels have been difficult to achieve without adverse effects. The minimal effects of quinidine, verapamil, ketoconazole, and cyclosporine A on nelfinavir's tissue:plasma
- 25 ratios are consistent with such low potency as demonstrated by their  $IC_{50}$  values relative to digoxin translocation across Caco-2 cells. By contrast, the compound of formula (II), which is at least 50-fold more potent than the other inhibitors, produced 75% to 90% inhibition of P-glycoprotein transport in both the brain and testes. This finding emphasizes the importance of potency in the application of P-glycoprotein
- 30 modulators.

                  Another issue of selectivity by currently available P-glycoprotein modulators is related to the inhibition of P-glycoprotein itself versus other membrane

transporters that may also be involved in drug efflux or drug uptake into the cell. An increasing number of both types of membrane transporters have been identified and characterized in various cells/tissues within the body. Moreover, cross-inhibition of different transports appears to occur. For example, a number of P-glycoprotein inhibitors such as quinidine, verapamil, ketoconazole, and valspodar also impair drug uptake by OATP, but at higher concentrations than those required for inhibition of the efflux transporter. See Cvetkovic, M., et al., OATP and P-glycoprotein transporters mediate the coordinate cellular uptake and excretion of fexofenadine, *Drug Metab. Disp.*, 27:866-871, 1999. Because an OATP type of transporter is present in the brain, (see Noe, B., Isolation of a multispecific organic anion and cardiac glycoside transporter from rat brain, *Proc. Natl. Acad. Sci.*, 94:10346-10350, 1997), it is not unreasonable to suggest that the observed reduction in nelfinavir's plasma ratio with higher doses of cyclosporine A reflects such non-selectivity. A similar effort with valspodar has also been observed with another P-glycoprotein substrate - digoxin. In contrast, since the brain:plasma ratio continues to increase over the whole dose range studied, compound of formula (II) does not appear to inhibit transporters other than P-glycoprotein, at least in the brain. See Dantzig, *supra*.

Thus, the present invention employs the 10,11-methanodibenzosuberanes of formula (I) to increase HIV protease inhibitor concentrations in the brain and testes, without an associated increase in plasma concentrations.

The 10,11-methanodibenzosuberanes of formula (I) are typically co-administered with an HIV protease inhibitor, such as nelfinavir, saquinavir, indinavir, ritonavir, or amprenavir. In one preferred drug administration protocol a patient is pretreated with one or more doses of a compound of formula (I), and another dose of the P-glycoprotein inhibitor is administered concurrently with a dose of the HIV protease inhibitor. Typically, HIV protease inhibitors are administered orally in tablet form three times per day, in amounts of 600 to 1200 mg per dose. Administration of the compounds of formula (I) can be via any accepted mode of drug administration.

Dosage levels of the compound of formula (I) for use in accordance with this invention range can vary according to patient condition and weight but

generally range from about 0.01 to about 50 mg/kg of patient body weight, more preferably about 0.1 to 10 mg/kg of body weight, and most preferably about 0.3 to 2.0 mg/kg of body weight per dose.

The administration of the compounds of formula (I) in HIV treatment  
5 protocols with protease inhibitors in accordance with this invention can be carried out using any pharmaceutically acceptable mode of drug administration. The compounds of formula (I) can be administered either alone or more typically in combination with pharmaceutically acceptable excipients, including those used in formulating solid,  
10 semi-solid, liquid, or aerosol dosage forms, such as, for example, tablets, capsules, powders, liquids, suspensions, suppositories, nasal solutions, aerosols or the like. The compounds of formula (I) can also be administered in sustained or controlled release dosage forms, including depot injections, osmotic pumps, biodegradable matrices, transdermal (including electrotransport) patches, and the like, for the prolonged  
15 administration of the compound at a predetermined rate, preferably in unit dosage forms suitable for administration of precise dosages. The compositions will typically include a conventional pharmaceutical carrier or excipient and a compound of formula (I). In addition, the present compositions may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc., including a suitable dose of an HIV  
20 protease inhibitor. Generally, depending on the intended mode of administration, the pharmaceutically acceptable composition will contain about 0.1% to 90%, preferably about 0.5% to 50%, by weight of a compound or salt of formula (I), the remainder being suitable pharmaceutical excipients, carriers, etc.

One manner of administration of the compounds of formula (I) is oral, using a convenient daily dosage regimen which can be adjusted according to patient  
25 condition and total antiviral treatment protocol. For oral administration, a pharmaceutically acceptable composition is formulated by the combination of a compound of formula (I) and optional protease inhibitor with any of the normally employed pharmaceutical excipients, for example, mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, sodium cross carmellose,  
30 glucose, gelatin, sucrose, magnesium carbonate, propylene carbonate, vegetable oils, or triglycerides, and the like. Such dosage compositions include solutions, suspensions, tablets, dispersible tablets, capsules, powders, lozenges, sustained release

formulations and the like. Preferably the compositions for oral administration will take the form of a tablet, capsule, or caplet.

Liquid pharmaceutical compositions in accordance with this invention can be prepared by dissolving, dispersing, etc. an active compound of formula (I) and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, glycols, ethanol, and the like, to form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting agents, emulsifying agents, or solubilizing agents, pH buffering agents and the like, for example, acetate, citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc.

Dosage forms or compositions containing active ingredient in the range of 0.005% to 95% with the balance made up from non-toxic carrier may be prepared. Other useful formulations include those set forth in U.S. Pat. Nos. Re. 28,819 and 4,358,603.

The present invention can also be carried out using formulations for parenteral administration, i.e, subcutaneous, intramuscular, intrathecal, or intravenous administration. Injectable dosage forms of this invention can be prepared as liquid solutions or suspensions, solid forms suitable for dissolution or suspension in liquid prior to injection, or as emulsions. Suitable excipient carriers are, for example, water, saline, dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, solubility enhancers, and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, cyclodextrins, etc. A more recently devised approach for parenteral administration employs the implantation of a slow-release or sustained-release system, such that a more or less constant rate of drug release is maintained. See, e.g., U.S. Pat. No. 3,710,795.

The percentage of active compound contained in such parenteral compositions depends on the specific use and the needs of the subject. However, percentages of active ingredient of 0.01% to 10% in solution are acceptable, and they may be higher if the composition is a solid which will be subsequently diluted to the

above percentages. Preferably the composition will comprise 0.2 - 10% of the active agent in solution.

### EXAMPLES

5                   The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as limiting the scope of the invention, but merely as being illustrative and representative thereof.

#### 10   Example 1

Inhibition of the P-glycoprotein transport pump was measured as a function of inhibition of digoxin transport in an *in vitro* culture system. Inhibition of digoxin transport was determined using a polarized monolayer of Caco-2 cells. Caco-2 cells were grown and cultured on 0.4 µm polycarbonate membrane filters as  
 15 described in Kim, R.B., et al., The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors, J. Clin. Invest., 101:289-294, 1998. Transport of [<sup>3</sup>H]-digoxin (15 Ci/mmol; Dupont-New England Nuclear, Boston, MA) was determined by its addition to either the basal or apical side of the polarized cell monolayer, and the transport over a four hour period of time of  
 20 radioactivity into the other compartment was measured in the absence or presence of putative inhibitor in both compartments. The extent of inhibition by each putative inhibitor was determined using the following equation:

$$25 \quad \% \text{ inhibition} = 1 - \left[ \frac{i_{B-A} - i_{A-B}}{a_{B-A} - a_{A-B}} \right] \times 100$$

where *i* and *a* are the percentages of digoxin transport in the presence and absence of  
 30 inhibitor, according to the direction of transport. IC<sub>50</sub> values were estimated from the Hill equation using the computer program Prism® (GraphPad Software Inc., San Diego, CA), and the data represent results obtained from at least 3 preparations on different days.

IC<sub>50</sub> values were calculated for various known P-glycoprotein inhibitors; ketoconazole (1.2 μM), cyclosporine A (1.3 μM), verapamil (2.1 μM) and quinidine (2.2 μM), were in the low micromolar range. Fig. 1 illustrates the P-glycoprotein inhibition observed with various other putative inhibitors. Nelfinavir exhibited comparable  
5 inhibitory potency (1.4 μM) to the potency of the known P-glycoprotein inhibitors. However, ritonavir (3.8 μM) and saquinavir (6.5 μM) were somewhat less potent, and the IC<sub>50</sub> value for indinavir (44 μM) was about an order of magnitude greater than the IC<sub>50</sub> values for the other HIV protease inhibitors. As shown in Fig. 1, the compound of formula (II) was by far the most potent of the P-glycoprotein inhibitors studied,  
10 with an IC<sub>50</sub> value (0.024 μM) over 50-fold lower than cyclosporine A.

### Example 2

The tissue distribution of nelfinavir in the absence of any other putative inhibitor was determined in *mdr1a*(+/+) and *mdr1a*(-/-) mice. Male *mdr1a*(-/-)  
15 mice (FVB/TacfBR-[KO]*mdr1a*N7), 6-12 weeks of age and genetically matched male *mdr1a*(+/+) mice (FVB/MTacfBR) weighing 20 to 30 g were obtained from Taconic (Germantown, NY). The animals were cared for in accordance with the USPHS policy for the Care and Use of Laboratory Animals and the experimental studies were approved by the Vanderbilt University Animal Care Committee.

20 The tissue distribution of [<sup>14</sup>C]-nelfinavir (8.5 mCi/mmol, Agouron Pharmaceuticals, Inc., San Diego, CA) was determined following intravenous injection (5 mg/kg) of an ethanol/0.9% saline solution over 5 minutes into a tail vein; the total volume injected was 4 μl/g. At specific times after drug administration and following anesthesia with isoflurane (Isoflo, Abbott Laboratories, Abbott Park, IL),  
25 blood was removed by orbital bleeding and the animal sacrificed. Subsequently, tissues were harvested, weighed, and homogenized with 4% bovine serum albumin solution. Total radioactivity was determined after the addition of 100 μl plasma or 500 μl tissue homogenate to vials containing 4 ml scintillation fluid (Scintiverse BD\*, Fisher Scientific Co., Fairlawn, NJ). The brain:plasma ratio was 0.06 in the  
30 *mdr1a*(+/+) mice, whereas the brain:plasma ratio was 2.3 in the *mdr1a*(-/-) mice. The distribution also varied in the testes, where the *mdr1a*(+/+) mice had a 0.29 testes:plasma ratio, and the *mdr1a*(-/-) mice had a testes:plasma ratio of 2:1.



Example 3

The effect of P-glycoprotein inhibitors was investigated in *mdr1a*(+/+) mice by pretreatment with equally divided doses given by intravenous tail vein injection, 30 minutes prior to and concurrently with administration of nelfinavir.

- 5 Inhibitors studied included the compound of formula (II) (2 x 0.5 to 25 mg/kg, Lilly Research Laboratories, Indianapolis, IN), verapamil (2 x 6.25 mg/kg, Sigma-Aldrich, St. Louis, MO) and quinidine (2 x 25 mg/kg, Sigma-Aldrich), each dissolved in 20% ethanol/0.9% saline; cyclosporine A (2 x 0.5 to 25 mg/kg, Novartis Pharma AG, Basel, Switzerland) dissolved in 10% ethanol/60% propylene glycol/30% water;
- 10 nelfinavir (2 x 25 mg/kg, Agouron Pharmaceuticals Inc., San Diego, CA), ritonavir (2 x 12.5 mg/kg Abbott Laboratories), saquinavir (2 x 25 mg/kg, Roche Products Ltd., Welwyn, UK), and indinavir (2 x 25 mg/kg, Merck Research Laboratories, West Point, PA) each dissolved in 10% ethanol/40% propylene glycol/50% 0.9% saline; and ketoconazole (2 x 25 mg/kg, Sigma-Aldrich) dissolved in 25% 0.2N HCl. All
- 15 drugs were injected in total volume of 4 µl/g and appropriate vehicle solutions were used in control studies.

- Similar tissue distribution studies were also performed to study tissue distribution of [<sup>14</sup>C]-saquinavir (9.8 mCi/mmol, Roche Products Ltd) and [<sup>14</sup>C]-indinavir (8.5 mCi/mmol, Merck Research Laboratories), using the compound of
- 20 formula (II) (2 x 25 mg/kg) as the P-glycoprotein inhibitor.

At least 3 mice were studied at each time point and differences in radioactivity between treated and control groups were analyzed by a two-sided Student's t-test with  $p < 0.05$  as the limit of statistical significance.

- As shown in Fig. 3, pretreatment with 25 mg/kg formula (II), 30
- 25 minutes prior to and concurrently with [<sup>14</sup>C]-nelfinavir, markedly altered the disposition of total radioactivity in *mdr1a*(+/+) mice. The brain concentration-time profile in particular was especially affected, as seen in Fig. 2. In untreated mice, radioactivity in the brain was more than 17 times lower than that in plasma with a mean brain:plasma concentration ratio of 0.06, based on the relative area under the
- 30 concentration-time curves. Formula (II) increased brain levels by 20-fold in contrast to those in the plasma, which only changed 2-fold. As a result, formula (II) treatment produced an 10-fold increase in nelfinavir's brain:plasma distribution ratio.

Subsequent studies, also illustrated in Fig. 3, based on tissue distribution measured two hours after nelfinavir administration showed that these changes are dose-dependent. Moreover, 10- to 15-fold higher brain levels could be achieved without affecting nelfinavir plasma concentrations at total dosages between 12.5 mg and 25 mg/kg. Comparison of these findings with those in *mdrla*(-/-) mice indicated that if all of the effects of formula (II) are accounted for by P-glycoprotein inhibition, then the transporter is inhibited by about 75% following a total dose of 50 mg/kg formula (II). Similar results were obtained with nelfinavir levels in the testes, with P-glycoprotein activity being inhibited by over 90%. Similar findings were also noted after intravenous administration of [<sup>14</sup>C]-saquinavir, [<sup>14</sup>C]-indinavir and pretreatment with 50 mg/kg formula (II).

More modest, though statistically significant changes, were produced by cyclosporine A, ketoconazole, and ritonavir administration, but these largely reflected increased nelfinavir plasma concentrations rather than altered tissue distribution. Finally, neither quinidine, verapamil, nelfinavir, saquinavir, or indinavir produced significant changes in nelfinavir's disposition at the doses studied. The results are summarized in Table 1:

Table 1: Tissue levels of radioactivity (ng/g tissue) in wildtype and *mdrla*(-/-) mice at 2 hr after intravenous injection of [<sup>14</sup>C]-nelfinavir (5mg/kg). Mice were treated with varying doses of formula (II) or other known P-glycoprotein inhibitors, 50 mg/kg (unless otherwise noted) in two divided doses, given 30 min prior to and simultaneously with [<sup>14</sup>C]-nelfinavir. Data are shown as mean  $\pm$  standard deviation.

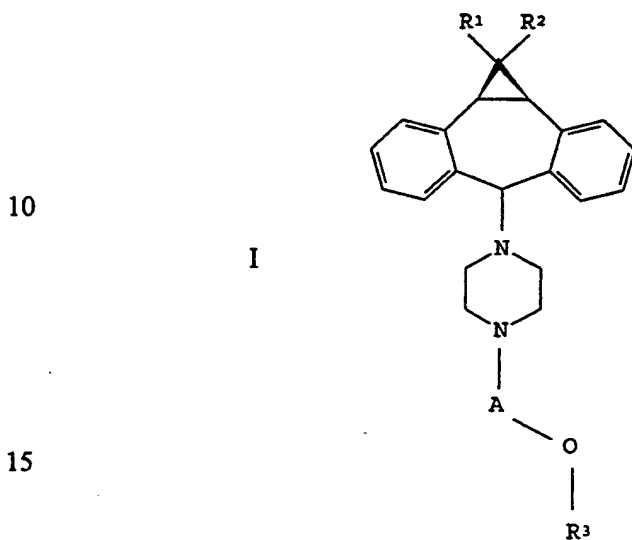
	Plasma	Brain	Brain:Plasma Ratio	Testes	Testes:Plasma Ratio
<i>mdrla</i> (+/+) Mice Vehicle Control	98 $\pm$ 12	5.1 $\pm$ 1.9	0.06 $\pm$ 0.01	31 $\pm$ 5.8	0.29 $\pm$ 0.02
Ritonavir (25 mg/kg)	618 $\pm$ 112	22 $\pm$ 3.8	0.08 $\pm$ 0.05	59 $\pm$ 3.4	0.31 $\pm$ 0.14
Nelfinavir (50 mg/kg)	124 $\pm$ 11	7.9 $\pm$ 1.5	0.06 $\pm$ 0.02	47 $\pm$ 6.6	0.39 $\pm$ 0.07
Saquinavir (50 mg/kg)	117 $\pm$ 14	6.9 $\pm$ 1.9	0.06 $\pm$ 0.01	48 $\pm$ 11	0.43 $\pm$ 0.13
Indinavir (50 mg/kg)	100 $\pm$ 6.2	7.5 $\pm$ 0.9	0.08 $\pm$ 0.01	37 $\pm$ 8.1	0.39 $\pm$ 0.05
Vehicle Control	99 $\pm$ 6.7	9.4 $\pm$ 3.0	0.10 $\pm$ 0.02	41 $\pm$ 6.8	0.38 $\pm$ 0.06
Quinidine (50 mg/kg)	92 $\pm$ 2.5	5.1 $\pm$ 1.5	0.06 $\pm$ 0.01	47 $\pm$ 7.3	0.54 $\pm$ 0.06
Verapamil (12.5 mg)	91 $\pm$ 6.1	8.4 $\pm$ 2.1	0.09 $\pm$ 0.02	39 $\pm$ 3.0	0.44 $\pm$ 0.06

		Plasma	Brain	Brain:Plasma Ratio	Testes	Testes:Plasma Ratio
	Ketoconazole (50 mg/kg)	292 ± 68	57 ± 14	0.20 ± 0.02	87 ± 16	0.30 ± 0.04
5	Cyclosporine Vehicle Control	103 ± 13	9.2 ± 1.2	0.10 ± 0.03	66 ± 12	0.42 ± 0.04
	1 mg/kg	120 ± 6.0	11 ± 2.4	0.10 ± 0.02	61 ± 7.0	0.51 ± 0.04
	4 mg/kg	322 ± 14	45 ± 20	0.13 ± 0.06	128 ± 10	0.40 ± 0.05
	12.5 mg/kg	698 ± 189	89 ± 19	0.18 ± 0.08	195 ± 54	0.30 ± 0.07
	25 mg/kg	659 ± 57	190 ± 43	0.30 ± 0.08	294 ± 50	0.44 ± 0.04
	50 mg/kg	954 ± 132	242 ± 46	0.27 ± 0.07	245 ± 55	0.25 ± 0.07
10	Formula (II) Vehicle Control	84 ± 4.9	6.6 ± 1.7	0.08 ± 0.02	47 ± 3.7	0.48 ± 0.07
	1 mg/kg	74 ± 14	9.4 ± 1.7	0.11 ± 0.04	56 ± 1.7	0.81 ± 0.13
	4 mg/kg	72 ± 4.8	24 ± 4.5	0.33 ± 0.04	95 ± 18	1.4 ± 0.33
	12.5 mg/kg	71 ± 11	60 ± 5.4	0.89 ± 0.16	108 ± 27	1.6 ± 0.44
	25 mg/kg	89 ± 8.1	89 ± 17	1.1 ± 0.28	168 ± 61	2.0 ± 0.48
	50 mg/kg	171 ± 12	243 ± 19	1.4 ± 0.08	187 ± 17	1.2 ± 0.19
20	mdrla (-/-) Mice Vehicle	89 ± 15	184 ± 20	2.3 ± 0.24	194 ± 34	2.1 ± 0.35
	Formula (II) (50mg/kg)	161 ± 24	318 ± 52	1.9 ± 0.12	207 ± 46	1.3 ± 0.13

Although the invention has been described in detail with reference to preferred embodiments, variations and modifications exist within the scope and spirit of the invention as described and defined in the following claims.

CLAIMS

1. A method for increasing the concentration of an HIV protease inhibitor in the brain of a patient, said method comprising administering to an HIV  
 5 infected patient an amount of a 10,11-methanodibenzosuberane of formula (I):

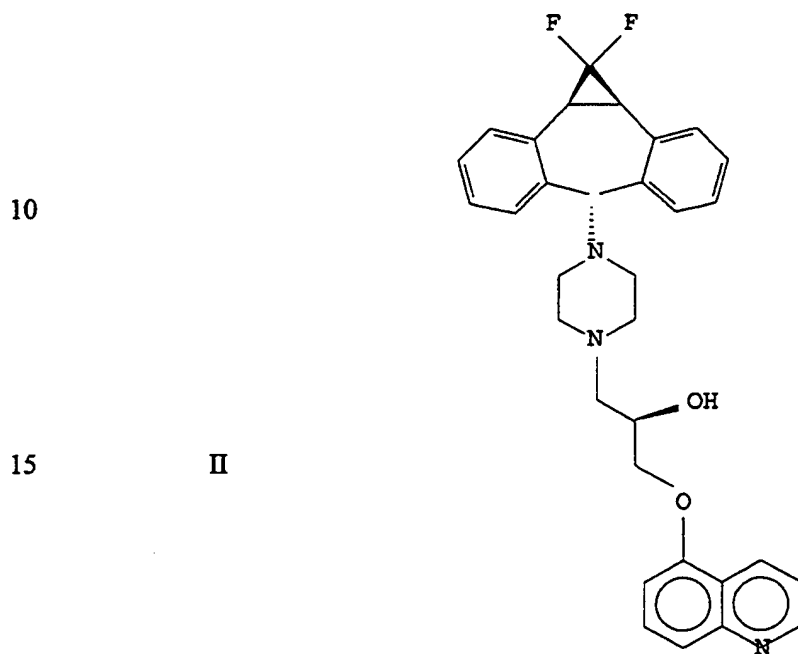


- wherein: A is -CH<sub>2</sub>CH<sub>2</sub>-; -CH<sub>2</sub>CHR<sup>a</sup>CH<sub>2</sub>- where R<sup>a</sup> is H, OH or lower acyloxy; or  
 -CH<sub>2</sub>CHR<sup>b</sup>CHR<sup>c</sup>CH<sub>2</sub>- where one of R<sup>b</sup> or R<sup>c</sup> is H, OH, or lower  
 20 acyloxy, and the other is H;  
 R<sup>1</sup> is H, F, Cl, or Br;  
 R<sup>2</sup> is H, F, Cl, or Br; and  
 R<sup>3</sup> is heteroaryl or phenyl optionally substituted with F, Cl, Br, CF<sub>3</sub>,  
 CN, NO<sub>2</sub>, or OCHF<sub>2</sub>; or a pharmaceutically acceptable salt thereof; and  
 25 co-administering to the patient a therapeutically effective amount of  
 the protease inhibitor.

2. The method of claim 1 wherein the patient is a male and the  
 concentration of the HIV protease inhibitor is also increased in the patient's testes.
3. The method of claim 1 wherein the protease inhibitor is  
 30 selected from the group of nelfinavir, indinavir, saquinavir, ritonavir, and amprenavir.
4. The method of claim 3 wherein the protease inhibitor is  
 nelfinavir.

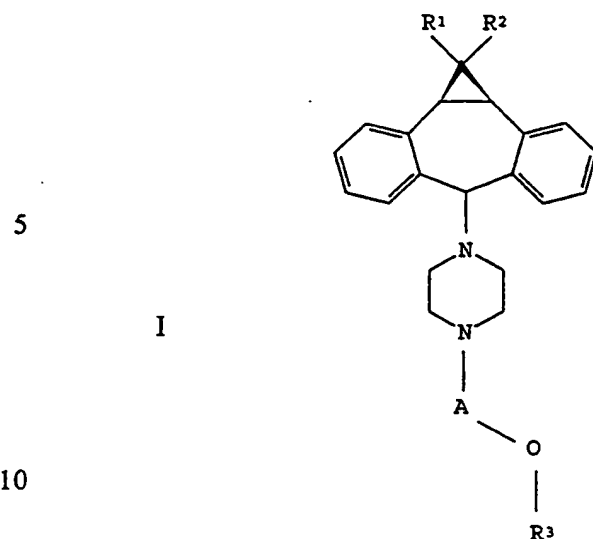
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5. The method of claim 1 wherein  $R^1$  and  $R^2$  are F, A is  $-\text{CH}_2\text{CHR}^3\text{CH}_2-$ , and  $R^3$  is optionally substituted quinolyl.
6. The method of claim 5 wherein  $R^3$  is OH and  $R^3$  is quinol-5-yl.
7. The method of claim 1 wherein the methanodibenzosuberane of formula (I) is a compound of formula (II):



8. A method of treating a patient having an HIV-1 infection comprising:
- administering to the patient a therapeutically effective amount of a protease inhibitor, and
- co-administering to the patient an amount of a compound represented by formula (I):

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wherein: A is -CH<sub>2</sub>CH<sub>2</sub>-; -CH<sub>2</sub>CHR<sup>a</sup>CH<sub>2</sub>- where R<sup>a</sup> is H, OH or lower acyloxy; or  
 -CH<sub>2</sub>CHR<sup>b</sup>CHR<sup>c</sup>CH<sub>2</sub>- where one of R<sup>b</sup> or R<sup>c</sup> is H, OH, or lower  
 15 acyloxy, and the other is H;

R<sup>1</sup> is H, F, Cl, or Br;

R<sup>2</sup> is H, F, Cl, or Br; and

R<sup>3</sup> is heteroaryl or phenyl optionally substituted with F, Cl, Br, CF<sub>3</sub>,  
 CN, NO<sub>2</sub>, or OCHF<sub>2</sub>; or a pharmaceutically acceptable salt thereof;

20 in an amount sufficient to increase brain levels of the protease inhibitor.

9. The method of claim 8 wherein R<sup>1</sup> and R<sup>2</sup> are F, A is  
 -CH<sub>2</sub>CHR<sup>a</sup>CH<sub>2</sub>-, and R<sup>3</sup> is optionally substituted quinolyl.

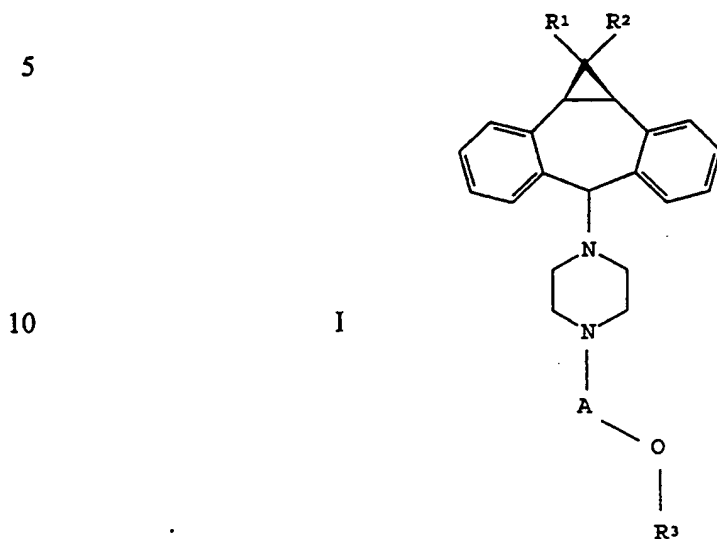
10. The method of claim 9 wherein R<sup>a</sup> is OH and R<sup>3</sup> is  
 quinol-5-yl.

25 11. The method of claim 8 wherein the amount of the compound of  
 formula (I) is sufficient to increase the brain levels of the protease inhibitor without  
 significantly increasing the concentration of the protease inhibitor in the patient's  
 blood.

12. The method of claim 8, wherein the amount of the compound is  
 30 also sufficient to increase concentrations of the protease inhibitor in the patient's  
 testes.

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13. A pharmaceutical composition comprising  
an antiviral protease inhibitor;  
a 10,11-methanodibenzosuberane of formula (I):



wherein: A is  $-\text{CH}_2\text{CH}_2-$ ;  $-\text{CH}_2\text{CHR}^a\text{CH}_2-$  where  $\text{R}^a$  is H, OH or lower acyloxy; or  
 $-\text{CH}_2\text{CHR}^b\text{CHR}^c\text{CH}_2-$  where one of  $\text{R}^b$  or  $\text{R}^c$  is H, OH, or lower  
acyloxy, and the other is H;

$\text{R}^1$  is H, F, Cl, or Br;

20  $\text{R}^2$  is H, F, Cl, or Br; and

$\text{R}^3$  is heteroaryl or phenyl optionally substituted with F, Cl, Br,  $\text{CF}_3$ ,  
CN,  $\text{NO}_2$ , or  $\text{OCHF}_2$ ; or a pharmaceutically acceptable salt thereof;  
and a pharmaceutically acceptable carrier therefor.

14. The composition of claim 13 wherein the  
25 methanodibenzosuberane of formula (I) is present in an amount effective to increase  
brain levels of the protease inhibitor.

15. The composition of claim 14 wherein the  
methanodibenzosuberane of formula (I) is present in an amount effective to increase  
brain levels of the protease inhibitor without significantly increasing plasma levels of  
30 the protease inhibitor.

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16. The composition of claim 13 wherein the protease inhibitor is selected from the group consisting of nelfinavir, indinavir, saquinavir, ritonavir, or amprenavir.

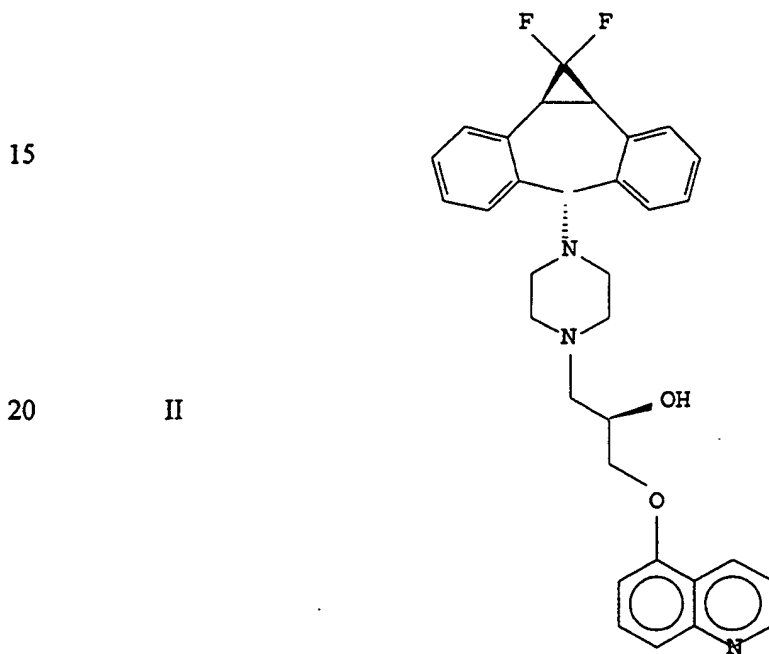
17. The composition of claim 16 wherein the protease inhibitor is nelfinavir.

18. The composition of claim 13 wherein  $R^1$  and  $R^2$  are F.

19. The composition of claim 13 wherein A is  $-\text{CH}_2\text{CHR}^*\text{CH}_2-$ .

20. The composition of claim 13 wherein  $R_3$  is a optionally substituted quinolyl.

21. The composition of claim 13 wherein the 10,11-methanodibenzosuberane is the compound of formula (II):

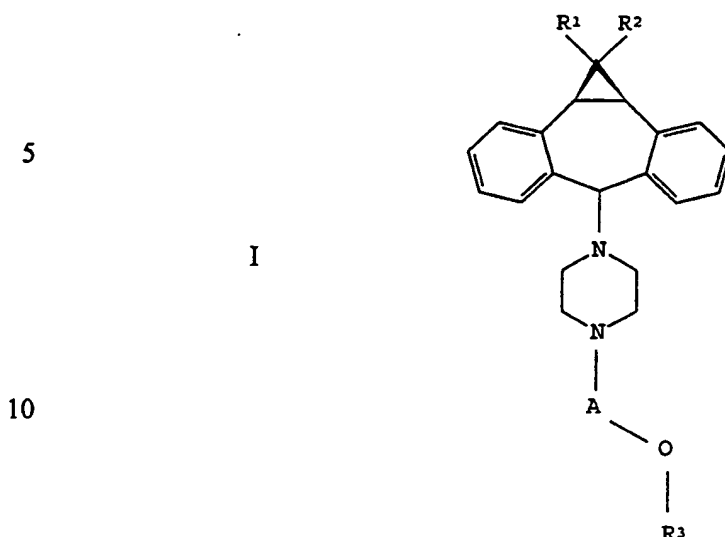


22. The composition of claim 13 wherein the methanodibenzosuberane comprises about 0.005 to 95% of the composition.



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23. Use of a 10,11-methanodibenzosuberane of formula (I):



wherein: A is  $-\text{CH}_2\text{CH}_2-$ ;  $-\text{CH}_2\text{CHR}^a\text{CH}_2-$  where  $\text{R}^a$  is H, OH or lower acyloxy; or  
 15  $-\text{CH}_2\text{CHR}^b\text{CHR}^c\text{CH}_2-$  where one of  $\text{R}^b$  or  $\text{R}^c$  is H, OH, or lower acyloxy, and the other is H;

$\text{R}^1$  is H, F, Cl, or Br;

$\text{R}^2$  is H, F, Cl, or Br; and

$\text{R}^3$  is heteroaryl or phenyl optionally substituted with F, Cl, Br,  $\text{CF}_3$ ,  
 20 CN,  $\text{NO}_2$ , or  $\text{OCHF}_2$ ; or a pharmaceutically acceptable salt thereof;

for the manufacture of a medicament for the treatment of HIV in a patient undergoing treatment with an HIV protease inhibitor.

24. The use of claim 23 for increasing the concentration of the protease inhibitor in the brain of a patient undergoing treatment with an HIV protease  
 25 inhibitor.

25. The use of claim 24 for increasing the concentration of the protease inhibitor in the patient's testes.

26. The use of any one of claims 23-25 for the manufacture of a medicament wherein the protease inhibitor is selected from the group of nelfinavir,  
 30 indinavir, saquinavir, ritonavir, and amprenavir.

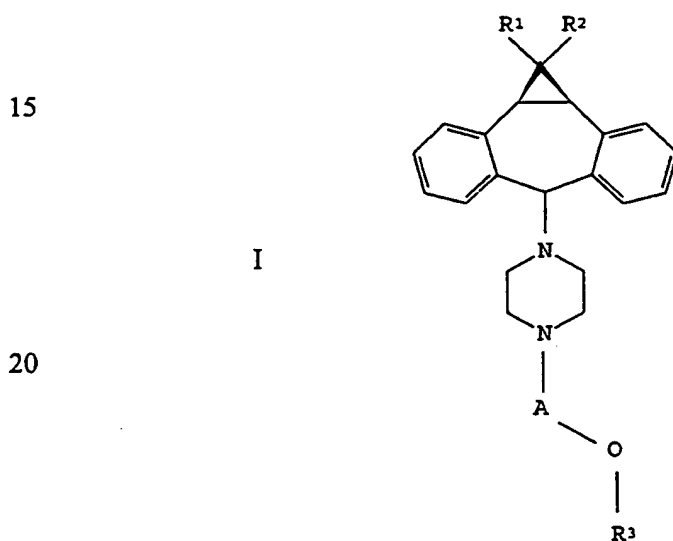
-24-

27. The use of any one of claims 23-26 for the manufacture of a medicament wherein  $R^1$  and  $R^2$  are F, A is  $-\text{CH}_2\text{CHR}^a\text{CH}_2-$ , and  $R^3$  is optionally substituted quinolyl.

28. The use of claim 27 for the manufacture of a medicament  
5 wherein  $R^a$  is OH and  $R^3$  is quinol-5-yl.

29. The use of claim 23 for the manufacture of a medicament for increasing brain levels of the protease inhibitor without significantly increasing plasma levels of the protease inhibitor.

30. Use of an HIV protease inhibitor for the manufacture of a  
10 medicament for the treatment of HIV wherein the concentration of HIV protease inhibitor in the brain is increased by co-administration with a 10,11-methanodibenzosuberane of formula (I):



25 wherein: A is  $-\text{CH}_2\text{CH}_2-$ ;  $-\text{CH}_2\text{CHR}^a\text{CH}_2-$  where  $R^a$  is H, OH or lower acyloxy; or  $-\text{CH}_2\text{CHR}^b\text{CHR}^c\text{CH}_2-$  where one of  $R^b$  or  $R^c$  is H, OH, or lower acyloxy, and the other is H;

$R^1$  is H, F, Cl, or Br;

$R^2$  is H, F, Cl, or Br; and

30  $R^3$  is heteroaryl or phenyl optionally substituted with F, Cl, Br,  $\text{CF}_3$ , CN,  $\text{NO}_2$ , or  $\text{OCHF}_2$ ; or a pharmaceutically acceptable salt thereof.

31. The use of claim 30 wherein the concentration of the protease inhibitor in the patient's testes is also increased.
32. The use of any one of claims 30-31 wherein the protease inhibitor is selected from the group of nelfinavir, indinavir, saquinavir, ritonavir, and  
5 amprenavir.
33. The use of claim 30 wherein the protease inhibitor is nelfinavir.
34. The use of any one of claims 30-33 wherein  $R^1$  and  $R^2$  are F, A is  $-\text{CH}_2\text{CHR}^A\text{CH}_2-$ , and  $R^3$  is optionally substituted quinolyl.
35. The use of claim 34 wherein  $R^A$  is OH and  $R^3$  is quinol-5-yl.
- 10 36. The use of claim 30 wherein the brain levels of the protease inhibitor are increased without significantly increasing plasma levels of the protease inhibitor.

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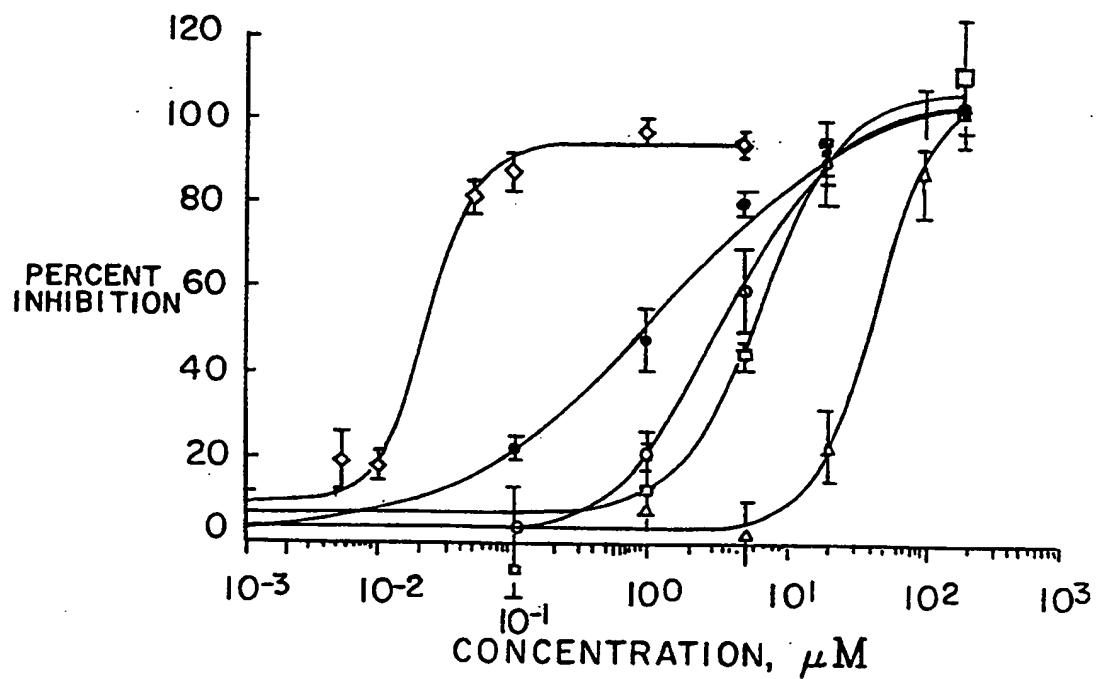


FIG. 1

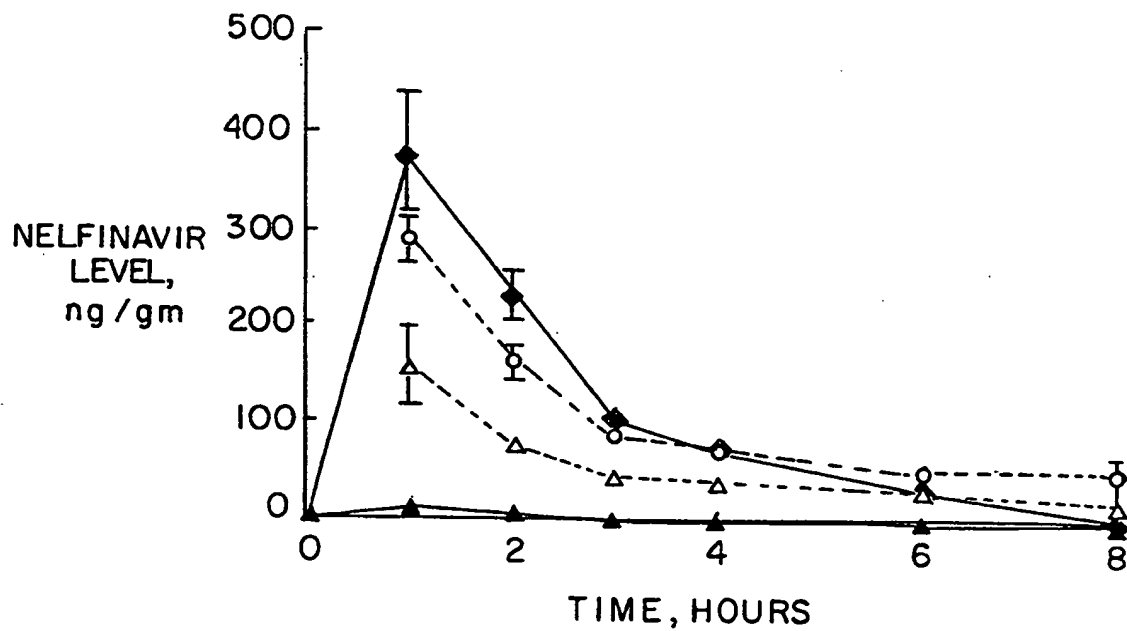
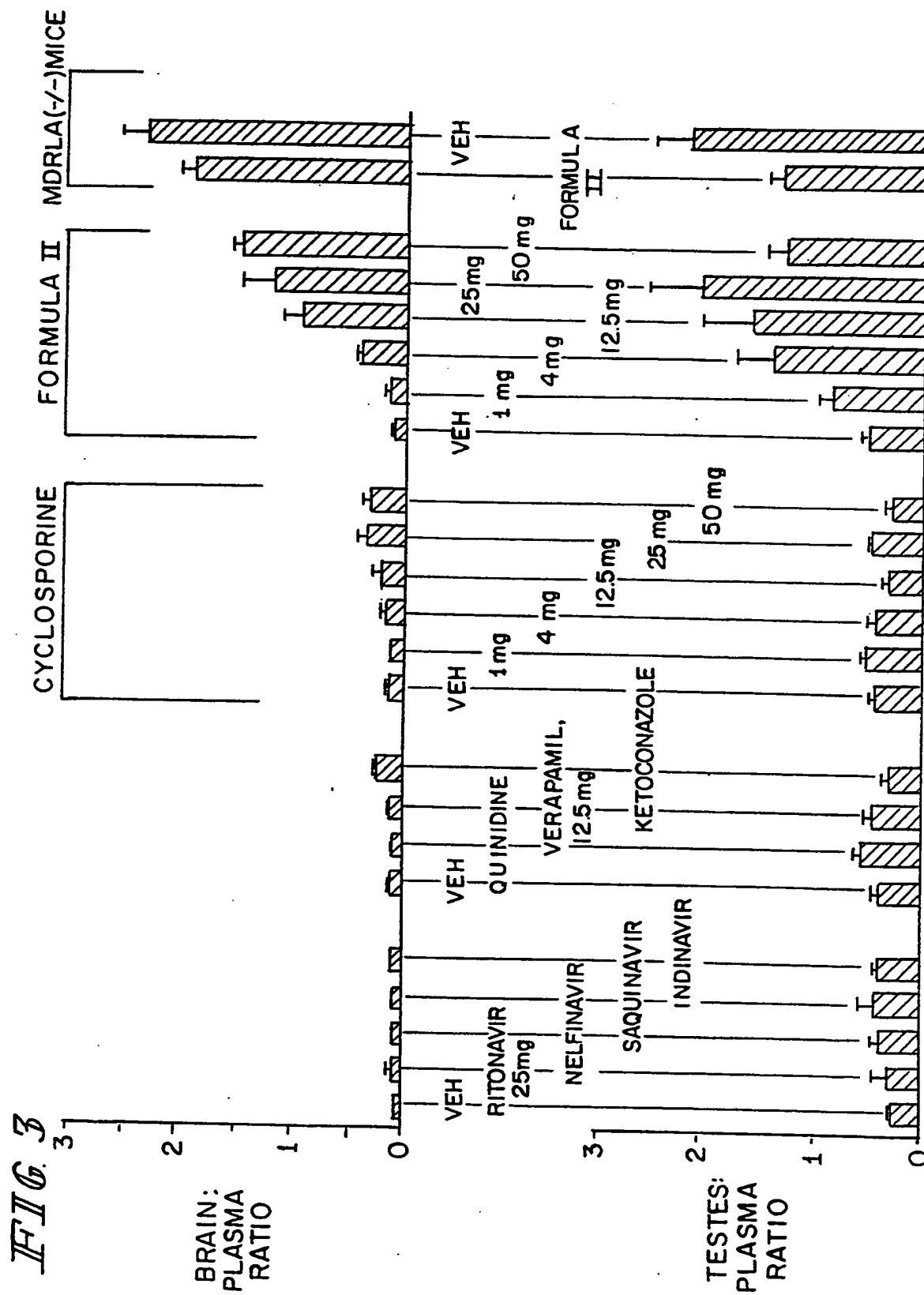


FIG. 2



## **Judy Schmidt**

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**From:** Anna W. Manville  
**Sent:** Wednesday, October 10, 2001 5:09 PM  
**To:** Judy Schmidt  
**Subject:** Park Marks

Judy,

we need a new file for the mark PARK INN INTERNTIONAL & Device (Class 16) in Japan. This mark has already been registered.

Also, new service mark application files in Japan for

PARK INN INTERNATIONAL (Class 42)  
PARK PLAZA INTERNATIONAL (Class 42)  
THREE TREE DESIGN (Class 42)

Please let me know what the numbers will be. Thx.